Eukaryotic Gene Expression: Basics and Benefits

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Module No. # 06

Lecture No. # 21

Regulation of gene expression by steroid hormones

Welcome to this lecture on the regulation of gene expression by steroid hormones. This is lecture number 21 in this course on eukaryotic gene expression basics and benefits. On the last few classes, may be last 5 classes, we have been discussing about how signaling molecules, which interact with membrane receptors, ultimately activate or repressed transcription of specific genes. That is how signaling molecules interact in cell surface receptor change or alter gene expression programs inside nucleus. We have been discussing at least 3 different kinds of receptors. One of them called GPCR or G protein coupled receptors which contain the 7 transmembrane domains and they what kind of signaling molecules interact with these receptors and what kind of signaling cascade ultimately leads to activation of gene expression.

We also talked about receptor tyrosine kinases. How growth factors binds to growth factor receptors, activates the receptors tyrosine kinases and how they through the map kinase activity lead to ultimately activation or repression of target genes. In the last class, we discussed about signal transducer and activator of transcription that is how starts or involved in the regulation of gene expression by cytokines. There are number of other cells of a signaling molecules and cells surface receptor pathways but we I gave you these 3 different pathways as an example of how molecules interacting with cell surface receptors can ultimately transudes signals through into the cell and activation of specific kinases can ultimately lead to the activation of specific transcription factors. These results in the activation or repression of gene expression and we also mentioned that there is lot of cross talk goes on between these different signaling pathways and I gave you some of the examples.

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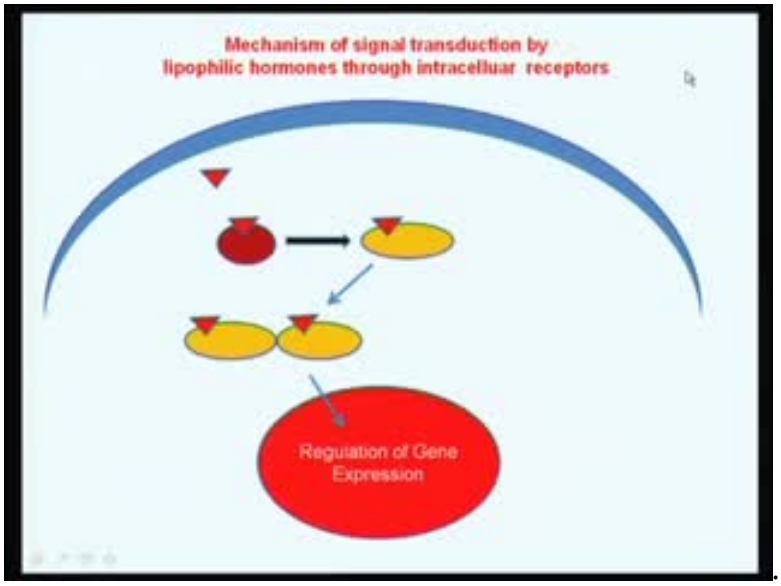

What we will do today? We will now go into the inside the cell and discuss about those molecules which can diffuse through the cell membrane and enter inside the cytoplasm and then bind to specific inter cellular receptors and how that results in the activation or repression of specific target genes. One group of molecules which do this or steroid hormones. So, what we will discuss in this class is how steroid hormones, what are steroid hormones and how steroid hormones enter the cell and bind to specific surfaces specific inter cellular receptors and activate or repress transcription of specific target genes? So, this is what is the I have shown a schematically here. So far, we have been discussing about molecules which bind to specific receptors on the cell surface but today we are now going to talk about molecules which can diffuse through the cell membrane, bind to specific receptors and often when these molecules bind to these receptors, it results in the conformational change.

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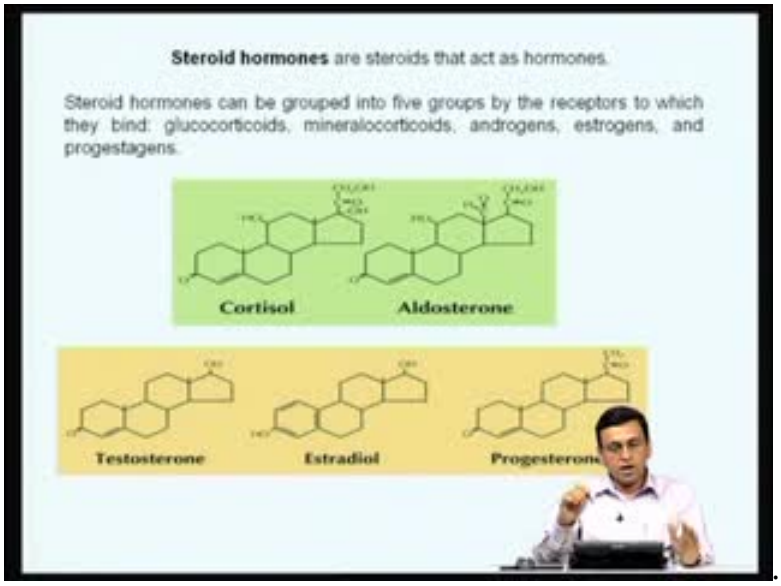

These modified receptors now often dimerize and this dimerize then goes inside the nucleus, binds to specific promoter sequences and regulates gene expression. So, we are going to use steroid hormones as example of this group of molecules and ask the question how steroid hormones are able to do this. That will be crux of today's lecture. What are steroid hormones? Steroid hormones are steroids that act as hormones. So, steroid hormones can be grouped into 5 groups based on the receptors through which they bind and these are known as glucocorticoids, mineralocorticoids, androgens estrogens and progestogens and I just given the structure of here cortisol is a glucocorticoid which is mainly made in the adrenal gland. Aldosterone is a mineralocorticoid again made in the adrenal gland. Testosterone is a male sex hormone which is made in the testes. Estradiol and progesterone are the female sex hormones which are made in the ovaries.

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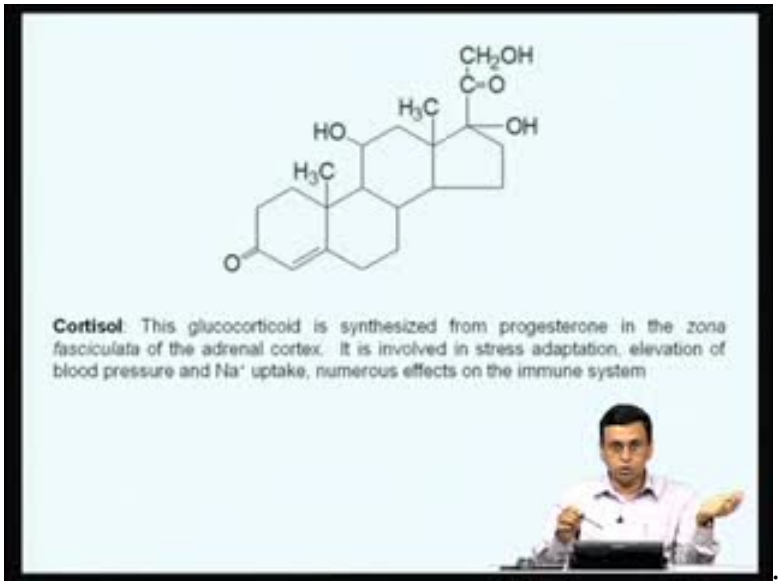

So, let us now and you can see now one type get tell you the name those are all very important molecules. They control a number of very important cellular processes and they all have more or less similar structure as you can see they are derived from the cholesterol. So, let us now try to understand how what are these molecule and how these molecules bring about their physiological effects? This is the structure of cortisol I have shown here. They are derived from cholesterol and this is a glucocorticoid which is synthesized from progesterone in the zona fasciculate of adrenal cortex. The adrenal gland has 2 major regions called the cortex and medulla. The glucocorticoids are made in the adrenal cortex whereas the epinephrine and non-epinephrine which we discussed in the previous class which are the molecules which interacts cell surface receptor, they are made from the adrenal medulla. Now, cortisol is involved in stress adaptation, elevation of blood pressure, sodium uptake and numerous effects of immune system including anti information. Many of the blocks which are available in the market reduce information or glucocorticoids.

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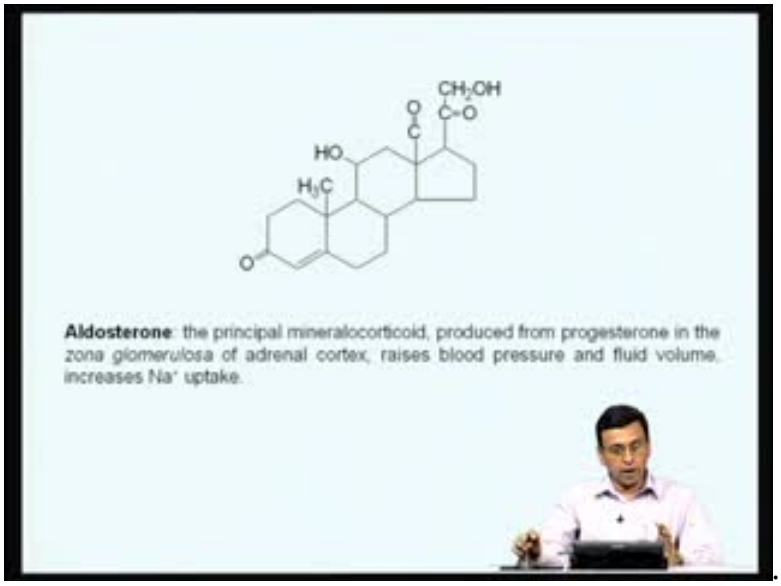

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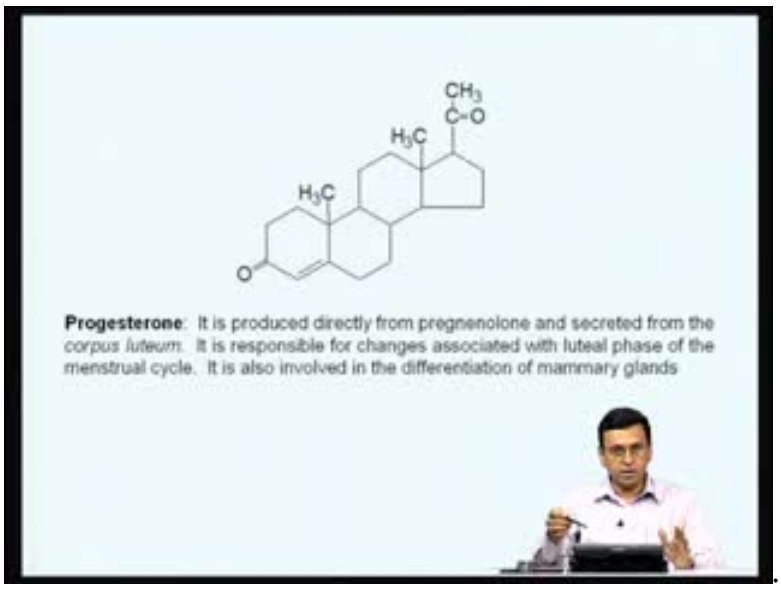

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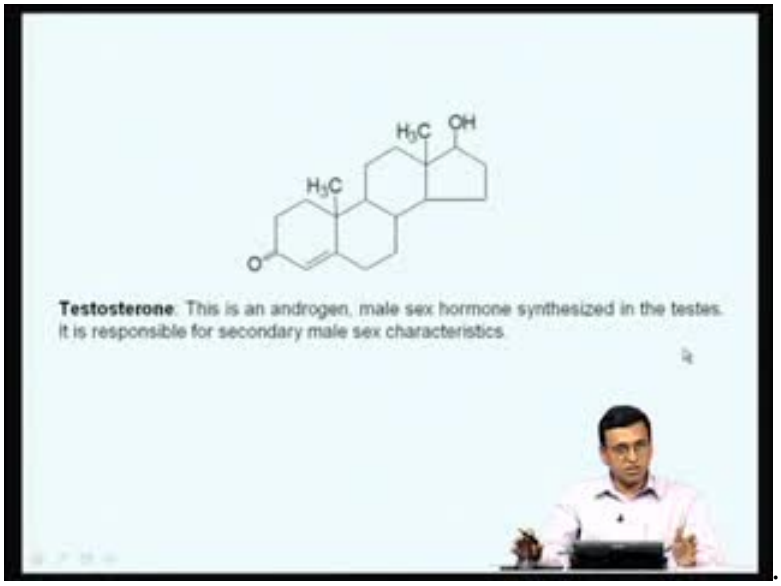

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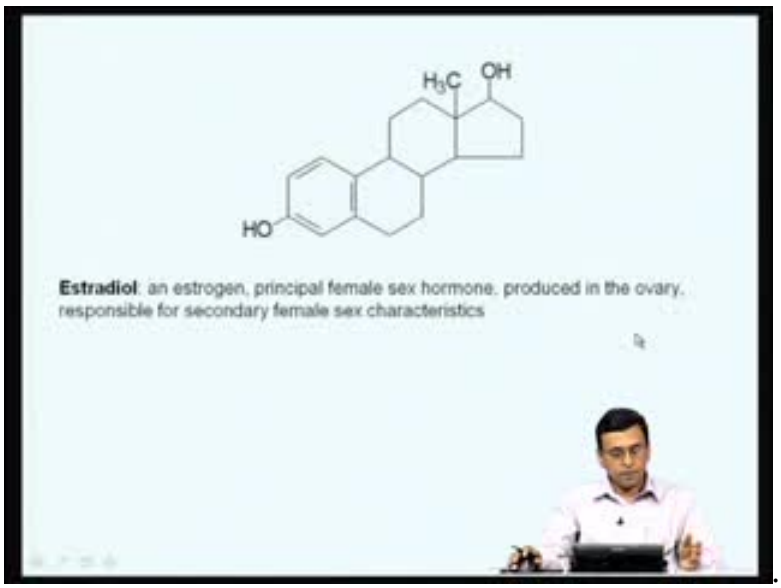

So, glucocorticoids act as major anti-inflammatory agents. All the steroids again, it is a mineralocorticoid. It is produced from progesterone again in the zona glomerular region of the adrenal cortex. Again, it is involved in the raising of blood pressure, adjusting regulation of fluid volume in the blood and also in the sodium ion uptake. So, it plays a very important role. Progesterone is a female sex hormone and it is produced directly from the pregenenolone and secreted from the corpus luteum of the ovary. It is responsible for changes associated with the luteal phase of the menstrual cycle and is

involved in the differentiation of mammary glands, a very important hormone in the case of females. Testosterone is a male reproductive hormone and it is an androgen. It synthesize in the testis and responsible for all the secondary sexual male sexual characteristics. Estradiol is again a female hormone, again produced from the ovary and is responsible for the female sexual characteristics.

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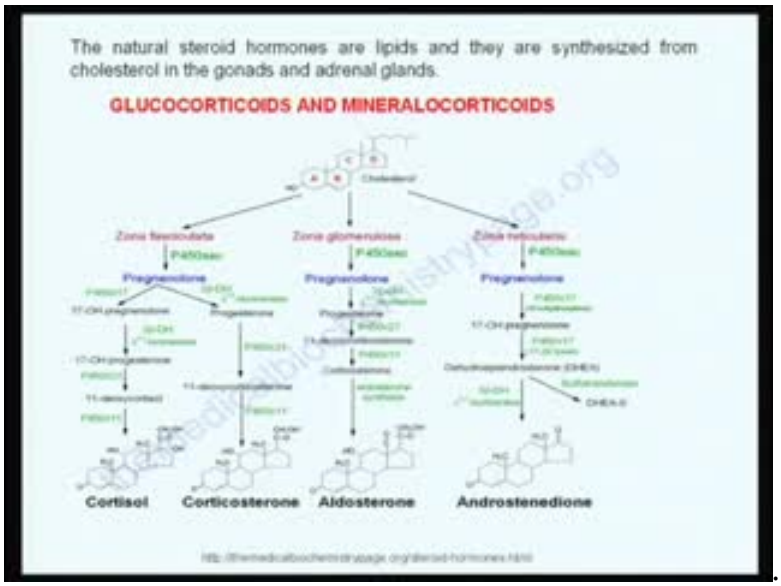

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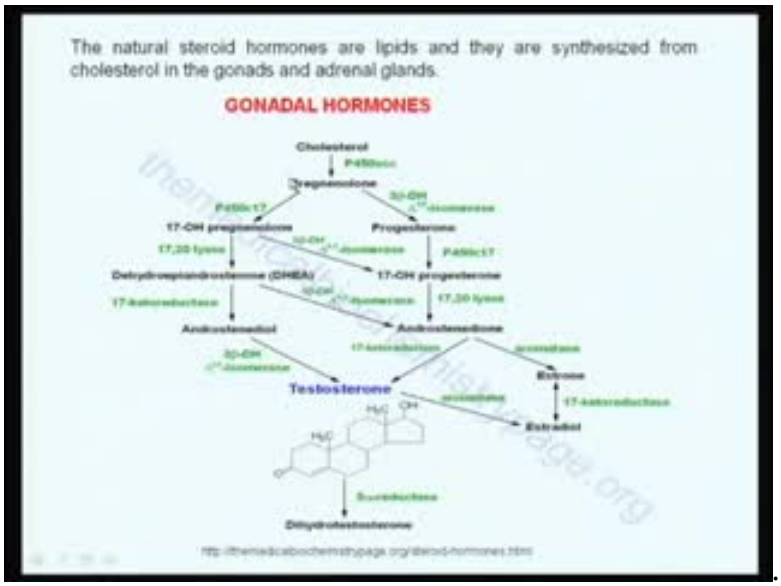

So, as you can see these 5 hormones are very important. They have very important biological functions and they are all synthesized from cholesterol. I have just shown here

a slide that is taken from this particular website which tells you how the glucocorticoids and the mineralocorticoids namely the cortisol, corticosterone together constitute the glucocorticoids. Aldosterone and androstenedione they together constitute the mineralocorticoids and how they are synthesized from cholesterol and what are the various enzymes involved in this pathway? Ultimately, these molecules are synthesized. We will not go in details of how they are made but just know that the glucocorticoids and mineralocorticoids, examples cortisol and aldosterone. They are synthesized by the admiral gland and they are all derived from cholesterol. Similarly, the gonadal hormones both Estradiol and progesterone as well as testosterone, again cholesterol is percussive for by synthesis and here is the pathway by which cholesterol is converted into the testosterone or estradiol. Need not remember the pathway at this stage but just to tell you that they all are structurally very similar molecules but they have very different physiological functions and they are produced in different regions of the body.

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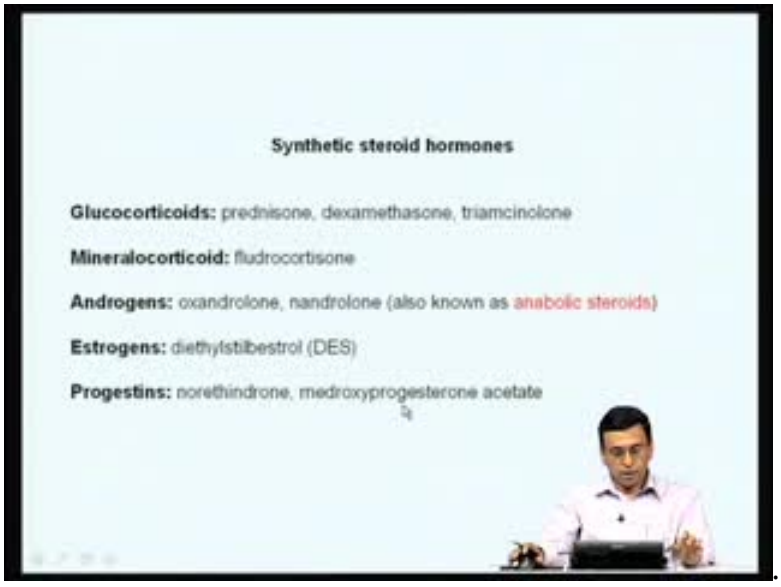

These are natural steroid hormone that we talk so far about. They are also what are called as synthetic steroid hormones again play a very important role, has lot of biomedical applications. There are variants of structure that you have just seen here and they have either more potent against or antagonistic action, mostly antagonistic action. The antagonist also has very important effects. Glucocorticoid synthetic glucocorticoid for example is called prednisone, dexamethasone, triamcinolone and so on and so forth. Synthetic mineralocorticoid fludrocortisones these are all derivatives of the basic

structure which I have shown in the previous slides. Many androgens in fact, they have very important role especially those athletics for example. Athletes who want to have more muscles who want to win races much faster; they take what are called as anabolic steroids which are actually banned.

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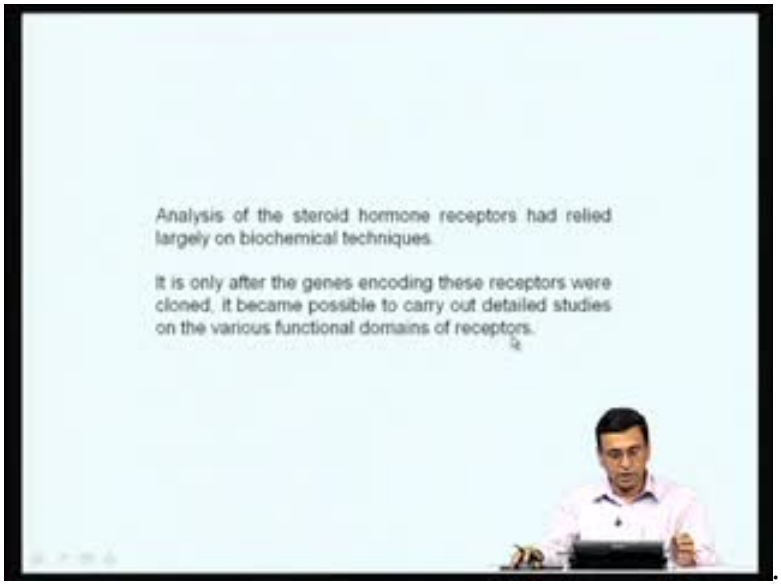

Athletes are not supposed to take these anabolic steroids, what is called as a dope test and if you test positive in this dope test, you cannot participate in many of these competition. These anabolic steroids are nothing but synthetic steroid hormones, especially synthetic androgens, oxandrolone, nandrolone and so on and so forth, very important in the sports area. Estrogens again have a synthetic estrogen, diethylstilbestrol called DES and you have synthetic progestins, non-ethindrone, medroxyprogesterone acetate so on so forth. So, these steroid hormones are very important biomedical applications because they are very portent molecules that alter the physiology. Now, some hormones have been around for a long time. In fact, steroid hormones have been known to exist since the early twenty century. However, it is only in the early in 1960s and especially in late 1980s, the molecular mechanism of action of the steroid hormones really became very clear.

So, it was only until, it is not until early 1960s that the idea of specific hormone binding molecules in the target tissue of these hormones began to emerge. So, till 1960, people did not know how these hormones are working but then when bio chemistry in early

1960s or 70s, the biochemistry ruled the world in the biology and when the biochemistry starts grinding the tissues and started looking what kind of protein molecules bind to this. Steroid hormones, it should become very clear tissues which are response to this hormone molecules actually contain proteins which bind to this hormones steroid hormones. So, the concepts that these hormones may be acting by actually binding to specific receptors began to emerge by bio chemical studies that carry carried out in the early 60s.

The analysis of the steroid hormone receptors had relied largely on bio chemical techniques as I said the one of the major methods by which people use to study steroid hormones, the late 1960s and early 1970s is you label, use a radio label steroid hormone and then you see how the radio label hormone goes and binds to protein that results and then try to monitor to the kinetics of binding and then see where this receptor is localize and so on and so forth.

So, primarily bio chemistry contributed a lot to understanding the molecules of the protein molecules which actually bind to the steroid molecules in the early 60s but it is only after the genes encoding these receptors were cloned, it became possible to carry out detailed studies of various functional domains of the receptor. So, the best that bio chemist could do is to see what kind of molecules bind and what is the affinity of this steroid hormones to these molecules and with a great difficulty they could actually purify some of this receptor molecules.

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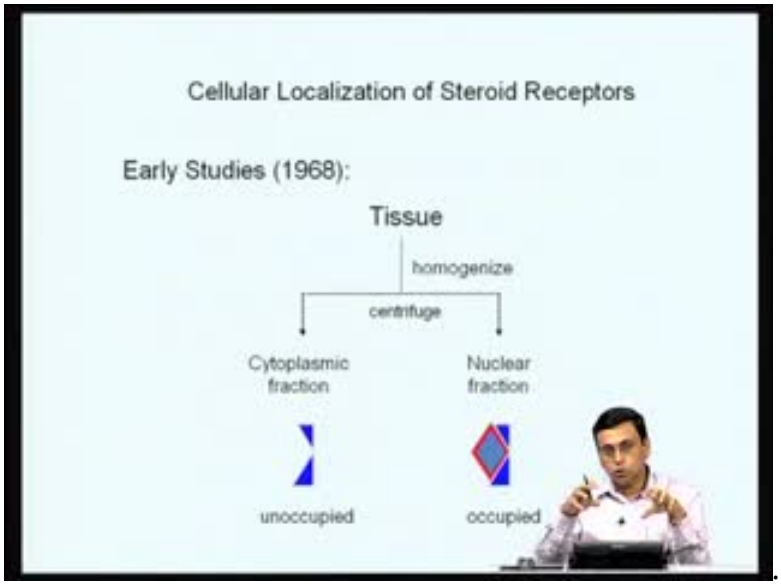

In late 70s some of these proteins or the receptor molecules could also be sequence, so the partial amino acid sequence of some of these steroid hormone receptors were available but complete characterization was not possible using this bio chemistry alone but the late 70s and the early 80s started was the drawn of molecular biology. It became to clone genes; it became to identify the amino acid sequence of proteins using the genes sequence so on so forth. Once restriction enzyme is cloning, technique became available that made a very major role in understanding the function structural and function of steroid hormone receptors.

So, what I am going to do is go through take you through a brief historical perspective of what kind of people are actually contributed a lot to the understanding of the steroid hormone receptors structure and function and what is the historical perspective of this filed. That is what we will discuss in next few slides. Now, as I said bio chemistry played a major role in understanding the structural function of steroid hormone receptors. For example, in the 1968 some of the studies actually led where you take a tissue, homogenize a tissue and then do a different gentrification by you can separate nuclei and you can separate a cytoplasm and then with they took this different faction and see where is the protein which is able to bind to this steroid hormone is present.

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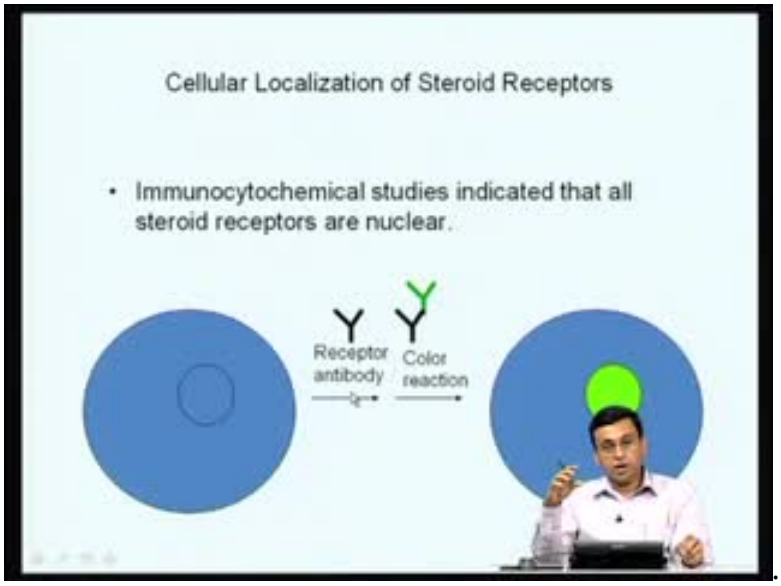

Based on such studies, people actually showed that when there is no hormone, this binding protein is actually present in the cytoplasmic fraction but the movement we have hormone, this activity sifted to the nucleus. So, this kind of bio chemistry started giving an idea of how these molecules actually act or how these molecules are able to bring about physiological response and they clearly told that these molecules actually enter inside the cell and binding to specific intracellular proteins. Again, once people started purifying using these kinds of technical localization studies and again using bio chemical purification techniques, people have started purifying this receptor protein from some of this tissue. Once you got a purified receptor, you could actually do a partial styptic digestion of this receptor or find out N terminal acid sequences or internal peptide sequencing and based on these peptides, as well as the purified receptor, you can immunize either mice or rabbits and you can generate antibodies.

So, once you have antibodies which are specific for this kinds of steroid hormone receptors, then people use this antibodies to ask the question where exactly this receptor are present. For example, if you have a specific tissue which has a binding protein for that particular steroid hormone, you do what is called immuno-fortis technique where you order receptor anti-antibody to specific that particular receptor. Then we have what is called the second antibody which is always conjugated to florescent molecule, it could be what has called florescent isothicyanate or (( )) and so many other modern dyes which under florescent microscope either give a blue colour, green colour or a red colour.

So, using this kind of immuno-fluorescence technique people and using receptor specific antibodies, people could actually demonstrate that these receptors when there is hormone inside the cell they are actually present inside the nucleus. So, picture began to emerge that these steroid hormones basically act by binding receptor molecules present inside the cytoplasm and once the hormone binds the receptor, this receptor is going to the nucleus and probably it is activating the transcription of specific genes.

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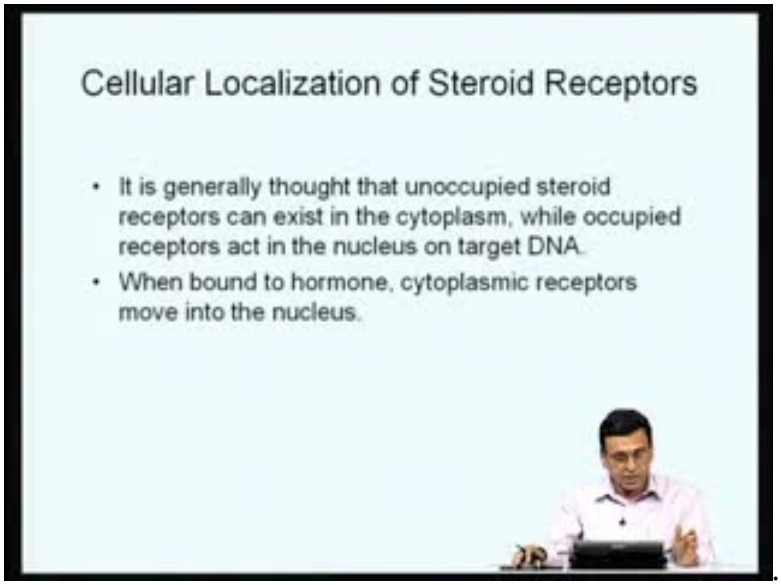

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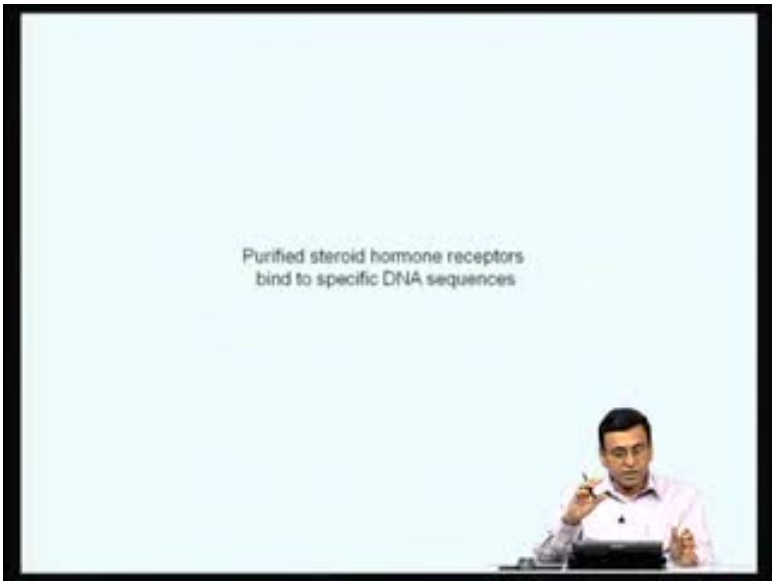

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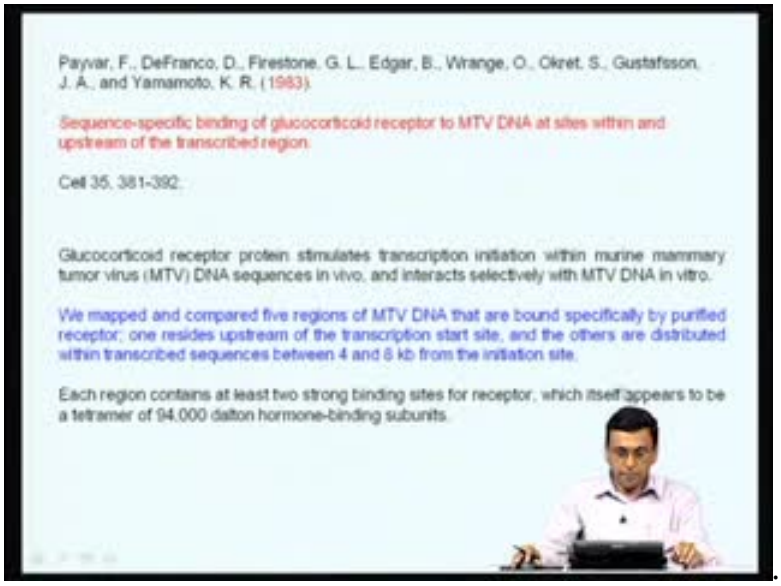

That is how these steroid hormones are able to bring out specific biological effects. This is the paradigm that began to emerge, so many of this bio chemical studies that was carried out in the late 1960s. So, this is what I have summarized. What I just told you here, it is generally thought that unoccupied steroid receptors can exist in the cytoplasm while the occupied receptors act in the nucleus on specific target DNA sequences but these are all again not much experimental evidences available, it is mostly conjunctional. So, when bound to the hormones cytoplasm, hormone receptors move to the nucleus and the purified receptors probably are going and binding to specific DNA sequences. Some of the key experiments which were done again using bio chemistry and little bit of molecular biology because as I said the late 70s and 1980s is the one that was the don of molecular biology.

A number of molecular biology techniques became available and people started using, especially bio chemist who were actually doing protein purification and protein characterize and technique now realize that they could answer many of this question using many of these molecular biology techniques by looking at the transcription by looking at the genes and so on and so forth. So, what for example Keith Yamamoto's group actually did in 1983. They published paper in cell in 1983, sequence specific binding of glucocorticoid receptors in MTVDNA at sites within and upstream of the transcribed region. So, that is virus called mouse mammary tumor virus and this virus response very well.

The trans for the transcription of this virus, you require glucocorticoids. So, people like Yamamoto started asking question, how does this mouse mammary tumor virus promoter is getting activated by glucocorticoids because glucocorticoids is producing in our body and here is a mammalian virus and the transcription of this virus is actually getting activated by a hormone which is produced by our body. So, what they did? They actually took what is called the long terminal region long terminal repeat or LTA of these particular MMTB virus or MTV virus and then ask the question where exactly does the glucocorticoid receptor binds because by this time, it is very clear.

All the effects of glucocorticoids are probably modulated to the glucocorticoid receptor. So, if the glucocorticoid is activating the expression of this mouse mammary tumor virus, people ask the question. The glucocorticoid receptor is must be doing something to the virus and the first question they ask this does it go and bind to specific DNA sequences within the mouse mammary virus tumor DNA. In fact, actually showed that there are about 5 regions within the MTVDNA, they are specifically bound by purified glucocorticoid receptor and one result upstream of the transcription start site and others are distributed within the transcribed sequences between 4 to 8KB from the initiation site. So, for the first time studies like this are actually shown that here is a receptor mammalian receptor that is actually binding to some specific sequences within this viral genome and this binding is probably responsible for activation of expression of the viral genes.

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So, such studies with purified steroid hormone receptors demonstrated that they are likely to be sequence specific DNA binding proteins and they are likely to be transcription factors. So, this is what emerged for many of these bio chemical studies. So, classical purificate, classical ligand, receptor interaction studies, understanding their kinetics of binding and affinity of this ligands to this receptors as well as bio chemical purification techniques followed by several localization using immuno-fluorescence as well as and more late 70s and early 80s using some of the molecular biology techniques, it became very clear that one of the major mechanisms by which these steroid hormones are acting is by binding to the steroid hormone receptors and these steroid hormone receptors are then going inside the nucleus and bind to specific regions within the DNA sequences. That is how they are able to bring out specific physiological effects. So, this is the paradigm that emerged before the molecular biology era started.

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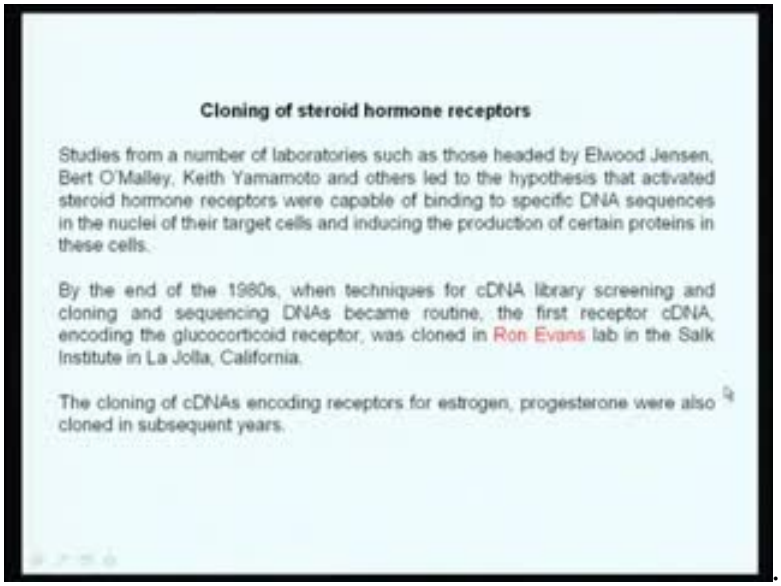

The most important phase in the study and understanding of the steroid hormone receptor function began with the cloning of steroid hormone receptors. So, I am going to give you a brief historical prospective of how this steroid hormone receptors were cloned. Now, studies from a number of laboratories such as those headed by Elwood Jensen, Bert O'Malley, Keith Yamamoto and many others, I am not going to quote everybody who worked in those areas these are some of the very important people who made very important contributions to the understanding of steroid hormone receptor functions.

All these studies actually led to the hypothesis that activated steroid hormone receptors go and bind to specific DNA sequences in the nuclear of the target cells and this is what induces the transcription of these genes leading to specific production of specific proteins. So, all the bio chemicals studies and immuno-localization studies and typical receptor ligand interaction studies, all these things pointed out that when you add a hormone to this steroid hormone to the cell, it is entering the cell, binding to this inter cellular receptors. Then it goes inside the nucleus and this binding of the receptor to specific DNA sequences results in the synthesis of specific proteins and that is how the steroid hormones are bringing about their physiological responses.

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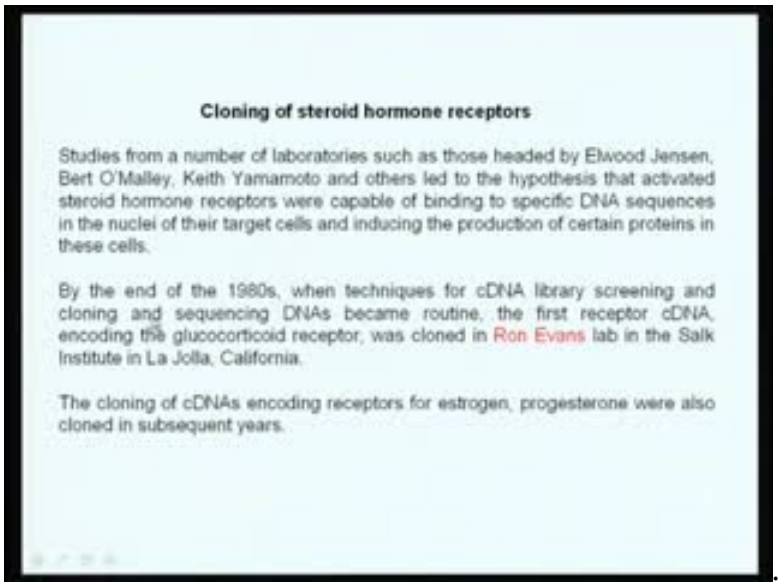

Now, by the end of 1980s when techniques for cDNA library screening and cloning and sequencing DNAs became a routine, the first receptors cDNA encoding the glucocorticoid receptor was cloned by Ronald and Evans in the Salk Institute in La Jolla California united states of America. So, 1980s as I told bond was the golden era of biology because the molecular biology techniques became available. People realized that you can actually purify messenger harness for a specific protein, encoding this specific protein and you can derive a cDNA of those and you can actually clone those DNAs. So, from millions and millions hundreds and thousands of genes which are available, you can actually pull out a specific gene or specific cDNA coding for a particular protein.

So, once the creation of cDNA libraries and how to screen these cDNA libraries and how to isolate the specific cDNA became available, people began to understand can we clone a cDNA that clones for or can we clone messenger RNA that actually syntheses this steroid hormone receptors. The first one was done by Ron Evans Salk institute in the late 90s the early 1980s. Once they receptors glucocorticoid receptor was cloned, this was soon followed by cloning of the cDNA encoding for estrogen, progesterone, androgen so on and so forth cloned in subsequent years. So, I am going to spend some to tell you how actually all these things were done because these are land mark papers in the area of steroid hormone receptor structure and function.

So, let us now see what exactly they did. So, the first paper on the characterization of the cDNA encoding the glucocorticoid receptor was published in journal nature in 1985-1986 by Ronald Evans Salk Institute and his entirely primary structure and expression of a functional Glucocorticoid receptors cDNA. These how would considered as a land mark paper in the area of steroid hormone receptors structure and function because this marked the beginning of a new era in the bio chemistry and this paved way are this probably is primarily responsible for our current understanding of the steroid hormone receptors structural function. So, what this group actually did is to demonstrate that there are 2 forms of glucocorticoid receptors cDNA's. So, what basically they did? They took the RNA isolating from a tissue which express a glucocorticoid receptor, converted them into cDNA by using reverse transcription and so on and then took the cDNA's of all these messengers or sensor in the tissue and then put them in the page vector and then made a library.

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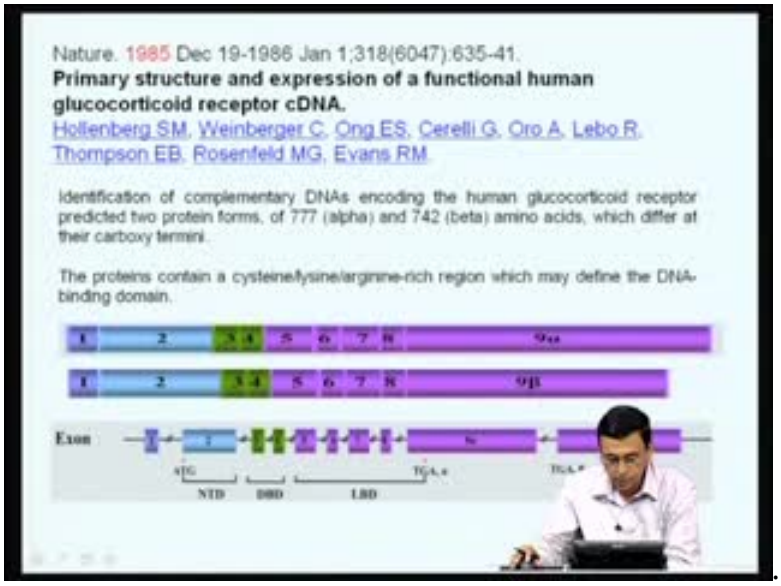

So, I have basically made a cDNA library and then using either antibody specific for glucocorticoid receptor or using oligonucleotides which specifically or digging the oligonucleotides that corresponds to the specific amino acid sequences of this glucocorticoid receptor which is available at that time because the purified receptor could be cleaved and the partial amino acids sequence could be identified for some of the peptides using either oligonucleotides that corresponds to those peptide sequence or by using antibodies rising purified glucocorticoid receptor. You basically, screen these libraries and ask the question which phase clone are for the cDNA that actually cores for the glucocorticoid receptor.

So, by cDNA screening, cDNA library screening they have actually identified 2 clones and one of them cloned for a glucocorticoid receptor of 777 amino acid and they called it as glucocorticoid receptor alpha. Another cloned for a 742 amino acid protein which they called as a glucocorticoid receptor beta and you can see this is the correct knowledge of glucocorticoid receptor structure and the foundation for this was actually laid in the year 1985-86 by Ronald Evans group. We now know that all these amino acids are encoded from about 9 hexons. This is the hallmark from 1 to 9 here and today, we know that the glucocorticoid receptor structures actually consists of what is called as a N terminal domain and it consists of a DNA binding domain which actually comes from hexon 3 and 4 and consists of what is called as ligand binding domain to which the glucocorticoid goes and binds. So, the alpha is after this whereas the beta is truncated here.

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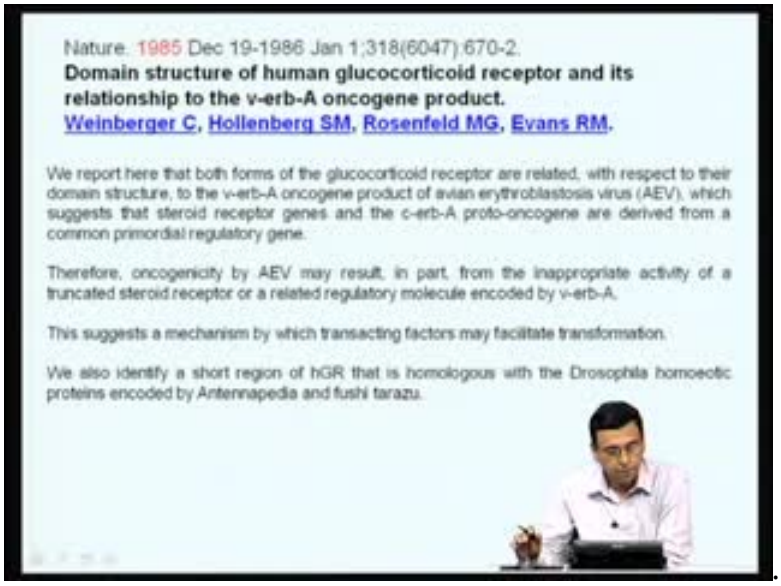

Now, in the same year from the same group, it also became another important land mark. Paper was published where it was reported that the domain structure of the glucocorticoid receptor and its relationship to the v-erb-A gene and its relation to v-erb-A oncogene product. Now, I can see here the importance of molecular biology.

Now, the bio chemical purification studies could only at the most partial amino acid sequence, all they could raise antibodies but nobody could obtain the full length amino acid sequence of glucocorticoid receptor using conventional bio chemical techniques because it is impossible for you to sequence the entire. How many amino acids? Almost 777 amino acids, you cannot do typical amino acids sequence of these proteins this 2 v-erb recurring a draw but once the cDNA sequences are available from the cDNA sequence; you can actually deduce the amino acid sequence and based on the amino acids sequence, people ask the question what kind of a protein is this? What kind of functional domains are actually present in this glucocorticoid receptor? Based on such studies in the subsequent paper they actually shown that this glucocorticoid receptor some of the domains actually resemble to that of an alkaline protein called v-erb-A.

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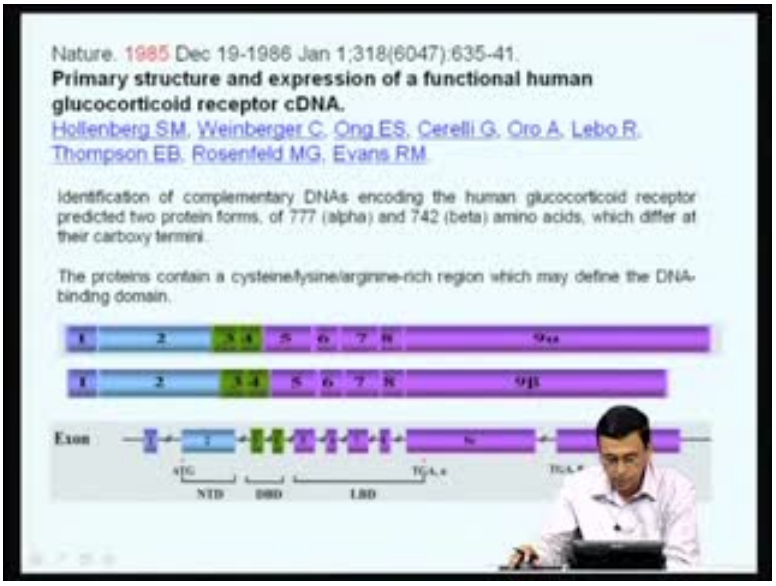

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Now, what is this v-erb-A? The v-erb-A is an oncogene product of a virus called avian erythroblastosis virus or AEV. So, these are viruses which when we infect, it actually causes cancer and one of the major proteins which is responsible for causing cancer in this virus is a protein called v-erb-A. What the studies of this group actually shown is that the structure of the glucocorticoid receptor very much resembles the structure of this oncoprotein. So, you can see people who have been studying steroid hormone, suddenly realize that there is some relationship between steroid hormone receptors and cancer because here is a viral protein which is causing cancer and the structure of protein is

resembles very much that that of that glucocorticoid receptor. So, people asked the question, what is the link? So, they predicted in this paper that the oncogencity of this virus may result in part from the inappropriate activity of a truncated steroid receptor or a regulatory molecule encoded by v-erb-A.

So, the movement they found that structurally these two proteins are related. People realised that the mechanism by which this oncoprotein is acting causing cancer, may be similar may be because of inappropriate signaling. So, they suggested that a mechanism by which transcription factors may facilitate transformation. So, these kinds of studies clearly showed that transcription factors may be involved in similar transformation leading to cancer and they also identified another short region in the human glucocorticoid receptor which had very high degree of homology to certain proteins which are involved in the regulation of development like The Drosophila homeotic proteins encoded by Antennapedia and fushi tarazu etcetera.

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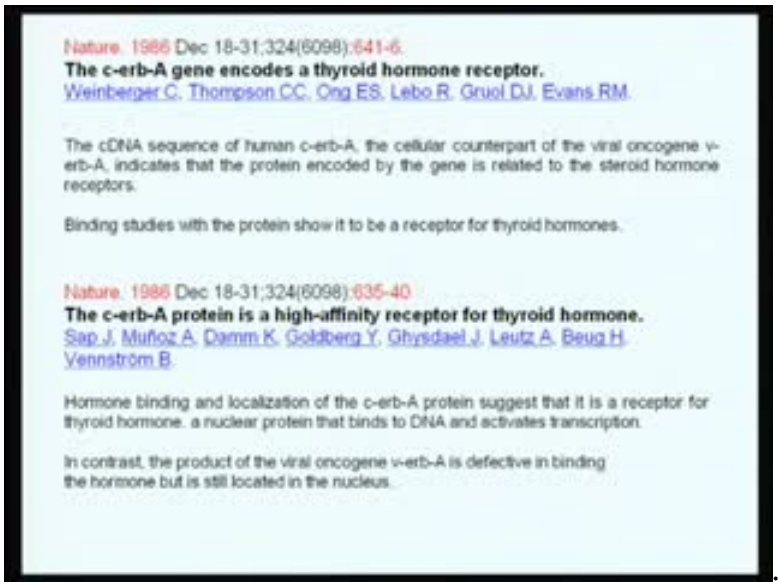

Now, we will come back to this transcription factor regulation in development sequence places but what I am trying to tell you is that once the cDNA for the glucocorticoid receptor was cloned and the domains structure was analyzed, it became very clear that this is like to be a transcription factor. There is something look like a DNA binding domain and it also had a homology to very important viral protein which causes cancer and people realized that the mechanism by which this virus are causing cancer may be

because of inappropriate activation of some of the genes which are actually activated by steroid hormone receptors. Now, they soon realized that the subsequent here 2 groups are actually published a paper where they actually demonstrate the v-erb-A gene is nothing but a thyroid hormone receptor.

So, the link between cancer and hormones became firmly established when they realized that one of the viral oncoprotein which very much resembles that of a glucocorticoid receptor actually course for a thyroid hormone receptors. I can see as I have just highlighted some of the important findings from this paper where this group, again from Ronald Evans lab actually showed the c-erb-A gene encodes a thyroid hormone receptor. What they showed is that the cDNA sequence of homology and c-erb human the c-erb-A the cellular counterpart of the viral oncogene, v-erb-A indicates that the protein encoded by the gene is related to steroid hormone receptors and binding studies with the protein show it to be a receptor for thyroid hormone.

So, they took the gene encoding for the thyroid hormone receptor, did what is called as invitro transcription and translation and that is you take the RNA, translate in the invitro and this invitro translate protein was able to bind to thyroid hormone. So, here is a viral oncoprotein involved in cancer that seems to thyroid hormone clearly saying that there is a link between the hormone signaling and cancer. Again, here in the same you can see these two papers are published in the same issue of nature back to back. This is from Vennstorm lab in Europe where again it is showed that hormone binding and localization of the c-erb-A protein suggest that it is a receptor for thyroid hormone, a nuclear protein that binds to DNA and activates the transcription.

So, the product of viral oncogene is defective in binding to the hormone but still localised to the nucleus. So, you can see here is a viral oncoprotein which binds to a DNA but it is not bind to thyroid hormone clearly indicating that if we have receptors which have defective in ligand binding or which are hormones binding and such receptors may cause inappropriate signal transition pathways activate inappropriate oncogenes. This is what can lead to ultimately cancer.

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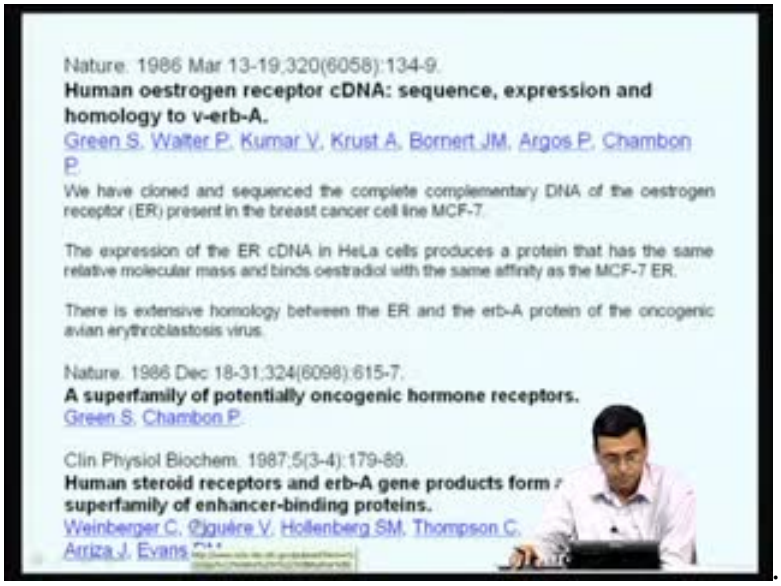

So, these kinds of studies established a link between viral virus induced cancers or a viral oncogene products and hormone signaling pathways. So, the cloning of glucocorticoids receptors are in the same time Ron Evans group clones glucocorticoid receptor. A number of other laboratory started cloning other receptors because they all had either antibodies of specific receptors or they had the partial peptides sequence amino acids sequence or salten peptides of this various receptors.

Pears Chambo group in France for example took the antibodies against the glucocorticoid estrogen receptor as well as they made ologo neutralize against peptide amino acid of specific peptides of the estrogen receptor and they cloned the estrogen receptors cDNA. So, they reported the cloning of the cDNA of estrogen receptors in the breast cancer cell line MCF-7 and expression of the ER cDNA in hela cells produces protein that has a same relative molecular mass and binds oestradiol with the same affinity as MCF-7 ER.

So, it became very clear that protein which is actually produced from this cloned gene is more over the same as the estrogen receptor that is expressed in a well-known breast cancer cell line. So, this they again they also reported that there is extensive homology between the estrogen receptor and the erb A erb-A protein of the oncogenic avian erythrocytosis virus. So, all these became clear that whether you clone glucocorticoid receptor, whether it is an estrogen receptor, they all seem to have similar structure and

the structure is very similar to that of an oncoprotein encoded by oncovirus or a tumor virus.

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So, all these studies clearly led to the proposal or hypothesis that there exists a super family of oncogenic hormone receptors. So, there are certain oncoproteins which are highly homologous to the hormone receptors and this super family of receptors may be actually involved in viral transformation. So, both Chambon as well as Roan Evan group, actually proposed that these human steroid receptors as well as some of this viral oncoproteins like erb-A may actually constitute a super family of enhancer binding proteins and they may actually cause cancer. Once the glucocorticoid receptor cDNA and estrogen receptor cDNA was cloned, subsequently number of other steroid hormone receptors was cloned. For example, within 1960s 1968 for example the chicken progesterone receptors cDNA was cloned as well as the human androgen receptor cDNA was cloned.

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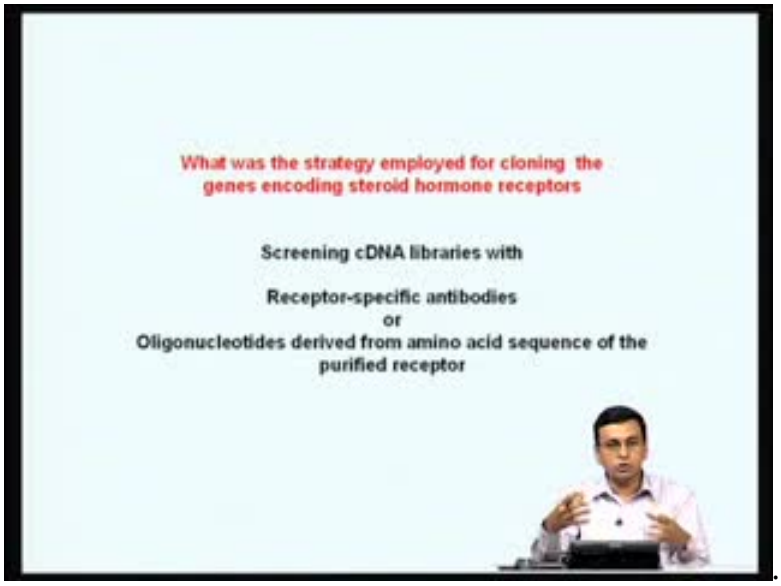

So, once all these cDNA for this steroid hormone receptors was cloned it became very clear that they all encoded super family of hormone receptors. This I am just putting a slide which I have already explained here. What was the strategy that was employed for cloning of all these genes including steroid hormone receptors? The strategy is to make a cDNA library that is you isolate messenger RNA from all these tissues which are expressing these specific receptors and then you convert all this messenger RNA into cDNA and then you clone this cDNA into phage vector and make a phage library or a phage DNA library.

Then take this phage cDNA library and you either probe them with antibodies against specific receptors which have been purified from various cell types. You take these antibodies against the purified receptors or make oligonucleotides degenerate oligonucleotides corresponding to the amino acid sequence of specific receptors and using either any labeled oligonucleotides or use antibodies, you can screen this phage libraries phage reading libraries. That is what they can isolate this cDNA clones and then you sequence this cDNA clone and reduce an amino acid from cDNA sequence and see what kind of a protein that they are coding for.

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So, this was a strategy that was used for isolating the various steroid hormone receptors in the early 80s and late 1980s. So, once all these studies were done, you have the receptor of glucocorticoid receptor, you had the receptor of estrogen receptor, thyroid hormone receptor so on and so forth. It became clear from all these studies that all these receptors had some common structural domain. For example, all these receptors have what is called as the ligand binding domain which probably binds the hormone. It is called as the hormone binding domain or the ligand binding domain. The receptors also has what is called as DNA binding domain in the middle and this probably responsive for binding specific sequences and it also what is called as amino terminal hyper variable region which was not that will consult between various receptors that then go so on and so forth. So, some of the three major functional domains of this receptors were a ligand binding domain, the DNA binding domain and hyper variable amino terminal domain. So, this is for the major domains which are present in all these receptors.

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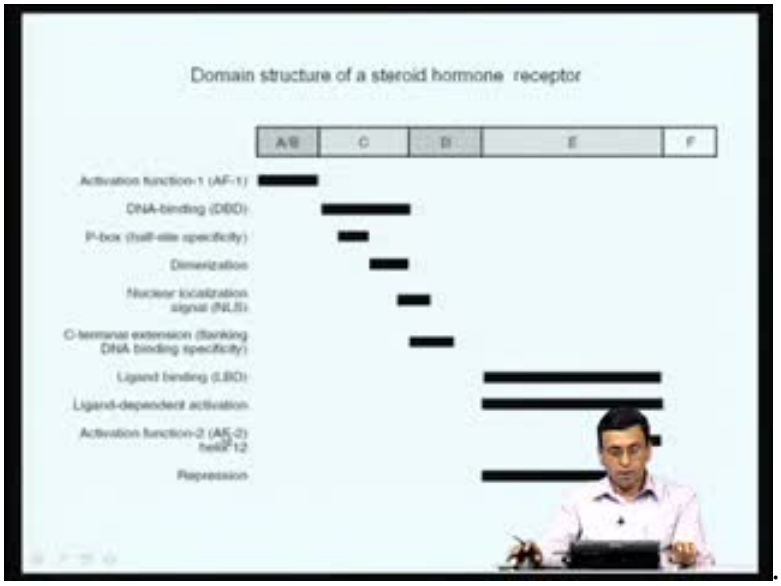

Today, we now know that there are much more detailed information available on the various domains of the steroid hormone receptors. For example, we now know that this amino terminal region which is actually shown as A slash B actually contains what is called as an activation function 1. This is actually responsible for ligand independent activation of steroid hormone receptors and we also know that the region known as C, actually course for a DNA binding domain and this DNA binding domains consist of 2 zinc fingers.

We have discussed about zinc fingers in the previous classes when we talk about the structure and function of various DNA binding proteins and this steroid hormone receptors contains 2C2H2 type of zinc fingers and we will look at this structure in a few more minutes. It also be very clear people started to looking at the mechanism by which this DNA binding domain acts while discuss in more detail in a few more minutes and this DNA binding domain what is called as the P box and what is called as a dimerisation region and plays a very important role in DNA binding function of the steroid hormone receptor.

As I said all these steroid hormone receptors are present in the cytoplasm when there is no hormone, they go into the nucleus in the presence of hormone. So, they contain what has called as nuclear localization signal and this nuclear localization signal is massed when there is no hormone once the hormone binds its induced conformational change.

So, the nuclear localization signal gets exposed. Therefore, receptor goes inside the nucleus.

Now, as was ligand binding domain, the ligand binding domain has two important functions. It has a ligand dependent activation function that means there is an activation function 2, whereas the one in the amino epidemic terminal referred as activation function 1. When ligand binds, this activation function is activated and now this in the presence of ligand, the receptors able to interact with the general transcription machinery and the RNA polymerize resulting in the activation of transcription. The domain also in the absence of ligand in certain receptor can act as a repressor. So, the cloning what of this cDNA's of these receptors paved way for a very detailed understanding of the various functional domains of these various steroid hormone receptors.

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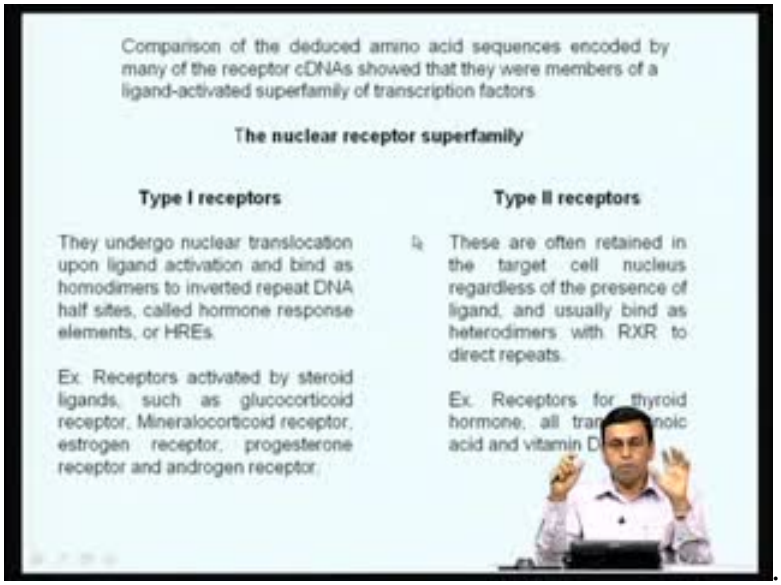

Now, once all these receptors were cloned, there also many it also became clear that the structure of this steroid hormone receptors made homology to other non-steroid receptors, for example thyroid hormone receptors. Thyroid hormone is not a steroid hormone but receptor for thyroid hormone was very homologous to then of the glucocorticoid receptor or estrogen receptor. So, people soon realized that there exists a nuclear receptor super family and this nuclear receptor super family contains not only steroid hormone receptors but also contains other non-steroid hormone receptors or other hormone receptors and which are not steroids. So, all these based on the structural

homology were grouped under one nuclear receptor super family. So, by sequencing cDNA's of various hormone receptors and deducing the amino acids sequences, all this were grouped under a single nuclear receptor super family.

Two broad categories of this receptors exists in this one are called as type 1 receptors, another called as type 2 receptors. The type 1 receptors, basically consists of steroid hormone receptors. They undergo nuclear translocation of the ligand activation and bind as homodimers to inverted repeat DNA half sites refer to as the hormone response elements. So, the DNA sequences to which the steroid hormone receptor binds or HRE or Hormone Response Elements. Glucocorticoid well bind to glucocorticoid response element, estrogen will binds to estrogen response elements, thyroid hormone bind to thyroid hormone response element so on and so forth. So, in general they are known as hormone response elements.

So, the example for the type 1 receptor are receptors activated by steroid ligands such as glucocorticoid receptor, mineralocorticoid receptor, estrogen receptor, progesterone receptor and androgen receptor. So, all these steroid hormone receptors are classified as type 1 receptors because usually they stay in cytoplasm in the absence of the hormone and once the hormone binds the conformational change, then going to the nucleus and often bind what is called as inverted repeated sequences. We will come to that in a minute whereas the type 2 receptors, they often are retained in the target cell nucleus regardless of the presence of the ligand. So, unlike classic hormone ligand receptors which are present in cytoplasm in a hormone and goes into the nucleus only when there you have hormone.

The type 2 receptors are already present into the nucleus, irrespective of whether the hormone is present in the hormone or not. So, they are nuclear receptors. Whereas, steroid hormone receptor cytosolic in nature in the absence of hormone and nuclear in nature in the presence of hormone and the type 2 receptor is always nuclear in the nature The function of ligand is actually to unfold or to activate the transcription activation function of the type 2 receptor. So, in the absence of hormone, although the receptor is bound to the DNA, it does not activate transcription but both the hormones binds; it induces a conformation change. Therefore, the receptor can activate transcription. Examples for type 2 receptors are thyroid hormone, retinoid acid, vitamin D so on and so forth.

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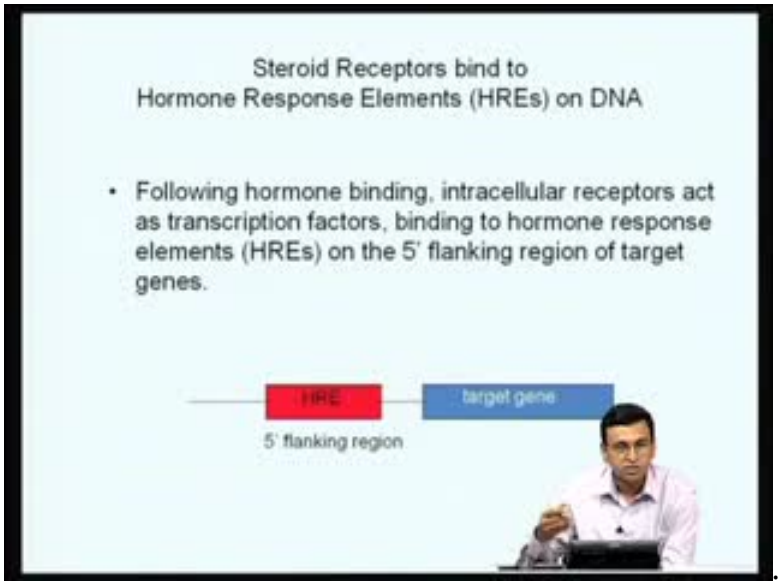

We will discuss type 2 receptors in the next class but today we will confine ourselves to only for the type 1 receptors which basically comprise of the steroid hormone receptors. Now, so it became very clear that these steroid hormone receptors act or bring about their physiological effects like primarily binding to what are called as the hormone response elements which are present on the promoter regions of various genes. So, once the hormone binds, the intercellular receptors now functions as transcription factors. They go and bind to the hormone response elements in the promoter regions of various genes and this is how they act as transcription of various genes. So, all the genes which are the target called glucocorticoid hormone. They invariably contain a hormone response element in the promoter region and that is how transcription of these genes are getting activated in presence of those hormones.

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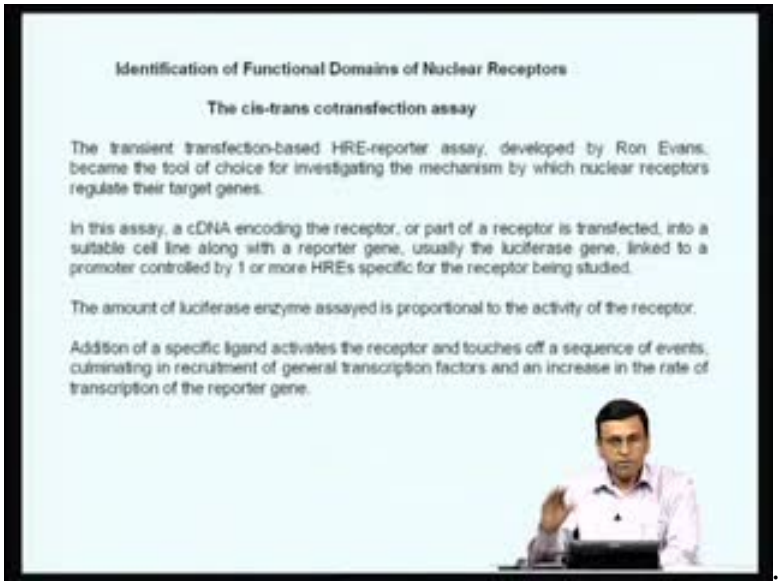

So, if the target gene needs to be activated by glucocorticoid, then you should contain glucocorticoid response element. If the product gene is to be activated by estrogen, it should contain an estrogen response elements and so on so forth. So, by having specific hormone response elements in the promoters of these target genes, these hormones by through their receptors are able to bind this in the sequences and bring about transcription activation. So, this became the general mechanism by which steroid hormones bring about transcription activation.

Now, one of the important experimental techniques that actually played a way for understanding the structural function of this nuclear receptors and the steroid hormone receptor is assay called as cis-trans cotransfection assay. It is because of this assay, it became possible to identify the various functional domains of these steroid hormone receptors. I have already discussed this cis-trans cotransfection assay in one of the earlier classes, especially the introductory classes of this course but I do not mind repeating again. Basically, what this actually means is that this is a transient transfection based hormone response element reporter assay developed again in the laboratory of Ronald Evans at salt institute and it became the tool of choice for investigating the mechanism by which nuclear receptors regulate their target of genes.

So, this is a very important assay and because of this assay that people could identify the various functional domains steroid hormone receptors. What is this in this assay? A

cDNA encoding the receptor or part of the receptor is transfected into a suitable cell line along with a reporter gene usually can be luciferase gene link to a promoter controlled by one or more of the hormone response element specific for the receptor being studied. So, basically what to do in this assay is that you take the cDNA that codes for, let me say I want to see whether glucocorticoid receptor actually activated from a promoter or not or whether a promoter actually contains a glucocorticoid response element or not.

So, what I do I take the promoter sequence or I take the sequence which suspected in the glucocorticoid response element and link it to a luciferase gene which is a reporter gene, it can also be (( )) or can be (( )) transferase, there are number of such reporter genes. So, take the luciferase gene, put it down stream of this suspected promoter which is response glucocorticoid and put this plasmin inside the cell. This is called as the cis factor.

Now, you take another plasmin which contains let us say for example a well-known viral LTR viral promoter which is known to be expressed by host transcription factor in a number of cell line. So, clone the receptor cell for glucocorticoid receptor under this promoter, so that when you put this plasmin inside the cell, the transcription factors present inside the cell go and bind to the promoter and they express the receptor. Once the receptor is expressed, the receptor will now go on and bind to the cis vector, the promoter region of cis vector and now if we add ligand, it will activate the transcription. So, if the cis plasmin contains the sequence for binding of the hormone receptor, then when the receptor is expressed from transplasmade, it will go and bind to luciferase vector and induce the express the luciferase gene. You can then you can measure in the luciferase activity and then you can now demonstrate that yes, this receptor can actually activate transcription activation from a specific region.

So, by using this assay not only you can identify hormone response elements in promoter region of any gene, you can also bisect out what region of the receptor is important. For example, you can make mutations by DNA binding domain and ask the question can it go and bind to DNA? Can it now activate transcription or you can change amino acid sequences within the DNA binding question and ask the question how is this specific domain or you can switch the ligand binding domain. For example, since I have DNA for estrogen receptor, have a cDNA glucocorticoid receptor, I can take the ligand binding domain of the glucocorticoid receptor and fuse it to estrogen receptor. Now, if I take the primary receptor and put inside the cell and since the glucocorticoid receptor, now has an

estrogen receptor cDNA, now it will activate transcription from glucocorticoid response element in response to estrogen but not in response to glucocorticoid. These kinds of experiment done using this assay clearly indicate that these steroid hormone receptors have a modulus structure.

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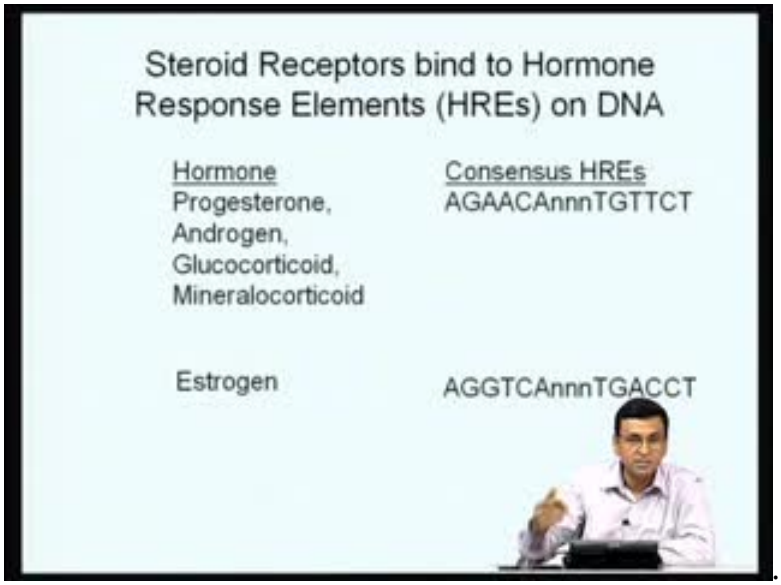

You can take the DNA binding group of glucocorticoid receptor and put it on estrogen receptor or you can take then DNA binding of estrogen receptor, put it inside glucocorticoid repeat and the deal is they start activating from each other response element that is the glucocorticoid response receptor contains estrogen response receptor DNA binding domain will now start activating transcription in estrogen response element or you can do other way also. You can take ligand glucocorticoid receptor binding domain or the ligand binding domain glucocorticoid receptor and switch it to that of the estrogen receptor. Now, they start activating transcription with each other ligand. So, these kinds of cis cotransfection assays, they would say for characterization of the DNA binding domain as well as ligand binding domain of the various steroid hormone receptors.

So, such studies actually demonstrated that steroid hormone receptors actually bind to hormone response elements and this actually led to the characterization of the various steroid hormone response elements. For example, hormone such as progesterone, androgen, glucocorticoid and mineralocorticoids, they all seem to bind to a specific the

same kind of response element containing AGAACA separate and TGTTCT separated by three nucleotides. This is actually called as an inverted repeat. You can see AGAACA and the opposite stands as TGTTCT, so AGAACA and the opposite stand also AGAACA and therefore is called inverted repeat. So, AGAACATGTTCT and the opposite stand again lead as AGAACA, so how this same AGAACA sequence on the two sides of the double DNA. So, these are called as palindromes or inverted repeat sequences. So, many of these steroid hormone receptors bind to the repeat sequences, it should become one of the hallmarks of hormone response element. Interestingly, all these four receptors progesterone, androgen receptor, glucocorticoid, mineralocorticoid, they all bind to a similar sequence which consists of what are called as two half sides.

The AGAACA is one half side, TGTTCT is another half side and these two half sides are separated by three nucleotides, it can be any nucleotides. So, when you have this kind of sequence in the promoter region of gene, you can say this is lightly to be activated by any one of these hormones. Similarly, if you take estrogen receptor, estrogen receptor actually binds to a sequence called AGGTCA, 3 nucleotides TGACCT. So, you can see the only difference between estrogen receptor or estrogen response element and a glucocorticoid response element is two nucleotides. So, if the sequence is AGAACA, it becomes glucocorticoid element instead of AGAACA. If it is AGGTCA, it becomes estrogen response element. So, you can see the physiological effects of estrogen is quite different from physiological effects of glucocorticoids but if you see at the molecular level, the only these two of the ways very fine difference that distinguishes between the estrogen receptors and progesterone receptor target genes.

So, they target gene promoter target genes, the promoter contains AGAACATGTTCT becomes responsible to glucocorticoid. If the same sequence instead of the 2A replaces such by AGT, it now become a response for estrogen receptors. So, this is a very fine difference exists at the molecular level between genes that are responsible for glucocorticoid and the genes that are responsible to estrogen and very fantastic experiments that actually done at the future characterized DNA binding through steroid hormone receptors. So, it became clear that these all these steroid hormone receptors whether it is GRMRPRE RRAR, they all contain two zinc finger domains and this zinc is coordinated by cystine-rich. So, they contain the C4 type of zinc fingers and it has been

very well shown that the DNA binding domain actually contains two very important regions called as P box and D box.

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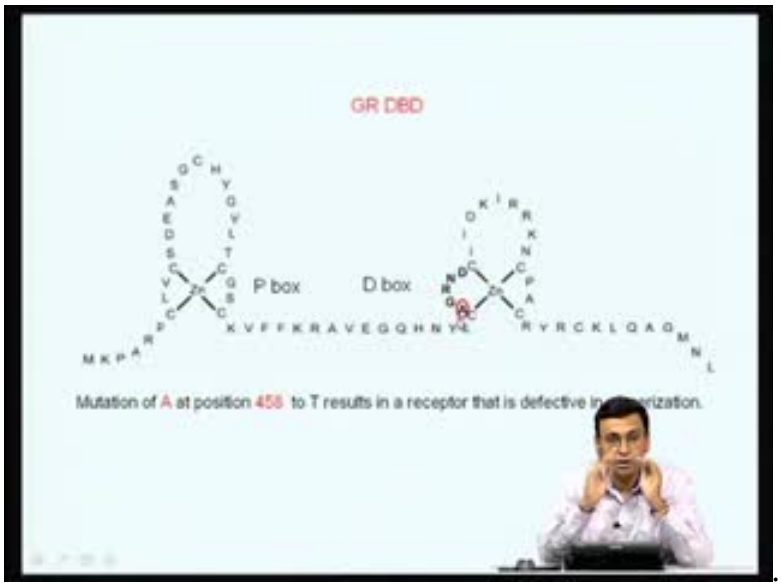

The D box refers to dimerise box or a distil box and P box is the proximal box and many number of people actually could do mutation within this the DNA binding domain and ask the question, what are the amino acids residues which are important for DNA binding. For example, if you now mutate this particular A adenine in this DNA binding alinine in this region. If you mutate that a quanine, now this receptor can no longer dimerize. So, if you mutate the IM residue that of (( )) in this amino acid position 458 in the glucocorticoid receptor to the glucocorticoid receptor cannot dimerize. So, can now just go back and tell you here, since the hormone response amino consists of palindromes that means you have two half sides AGAACA1 on one stand and AGAC on other stand. Therefore, the steroid hormone receptors in order to activate transcription and they go on and bind as dimers.

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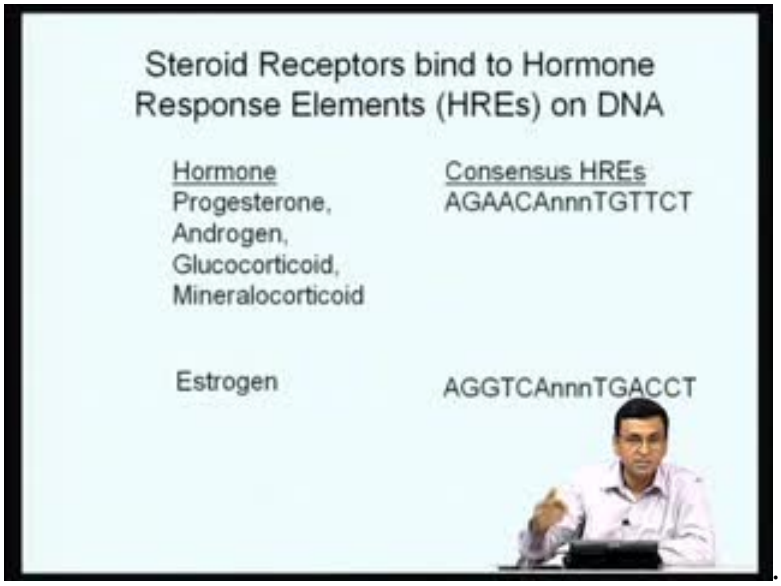

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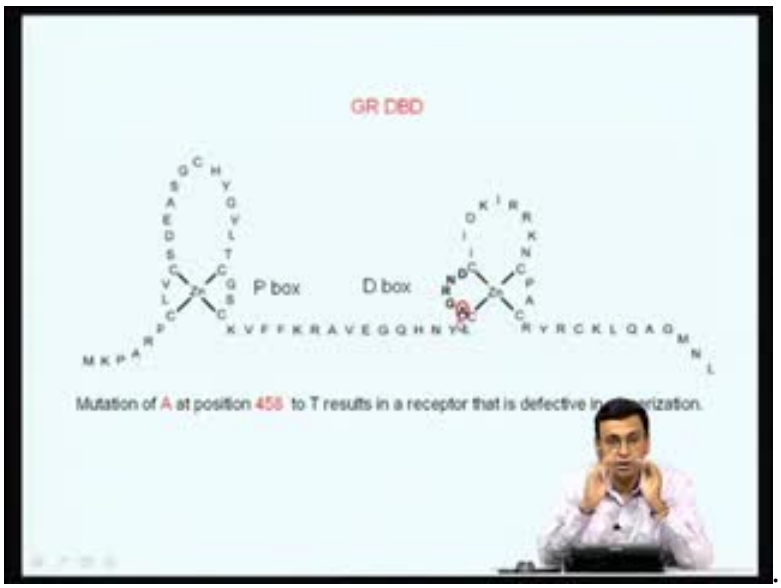
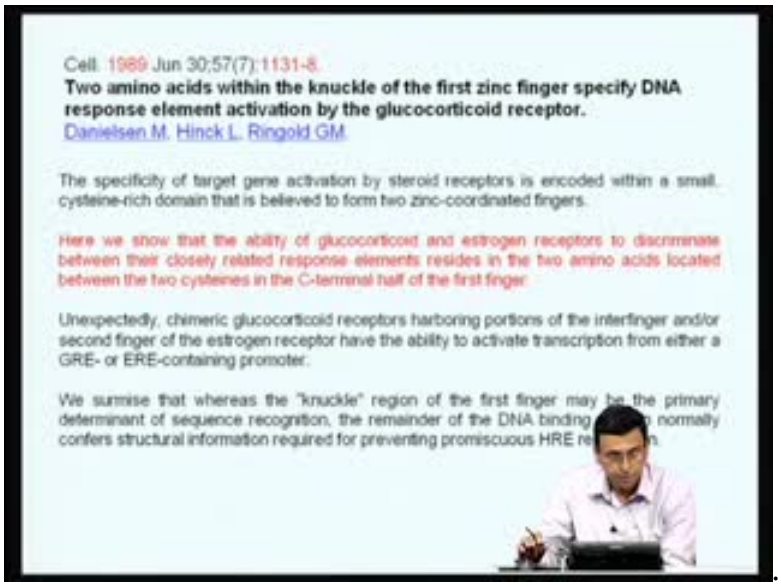


So, one hormone recognizes its one half sides on one stand and the other hormone recognizes on the other stand. In order to follow to bring together, we need a dimerisation domain. There is a dimerisation interface domain and there is also dimerisation interface in the ligand binding domain of all these steroid hormone receptors. So, using this kind of cis-trans cotransfection assay people have to identify what are the important amino acid residues that play very important role in DNA binding. Like I said in the case of glucocorticoid receptor, one mutation actually can

abolish the dimerisation function of the receptor. A very important experiment that was done in the 1989 in the year 90 by the two groups, one headed by Gordon Ringold lab as well as Ron Evans lab in the Salk Institute, they actually demonstrated by making specific mutation within this DNA binding domain, you can actually change this specificity of one receptor to other.



For example, in this paper published in cell where they actually showed two amino acids within the knuckle of the first zinc finger specify DNA response element activation by the glucocorticoid receptor. What they actually showed in this paper is that the ability of glucocorticoid and estrogen receptors were discriminated between the close related response element results in two amino acids located between the two cysteines in the C-terminal half of the first finger.

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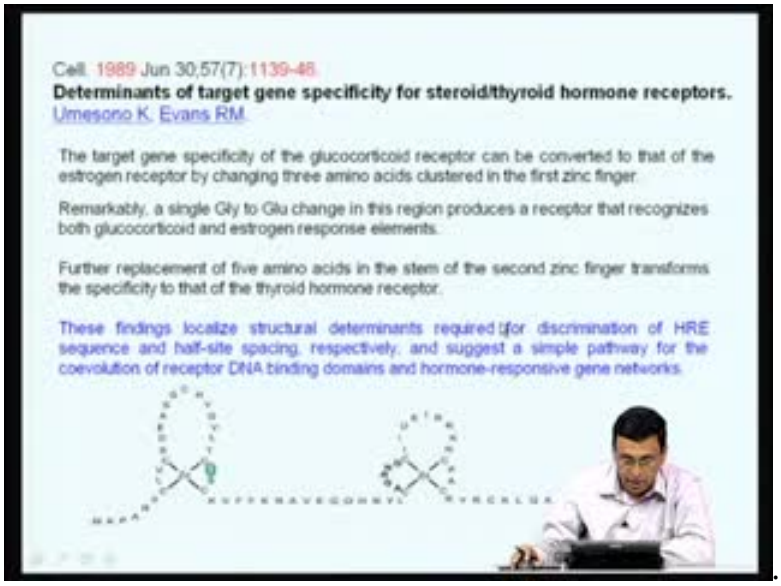

I have told you glucocorticoid response element is AGAAC half side whereas the estrogen receptor instead of AGAACA it is AGGTCA. So, only two nucleotides are different. What is paper actually include is that if you change two amino acids in the DNA binding domain of the glucocorticoid receptor is that to recognize that glucocorticoid receptor is now recognized to that receptor. A similar paper was published in the same issue of cell and this is the amino acid by again from the Ron Evans group. Again the same issue of cell where actually showed as that simply change in rising residue to glutamate in the base of the first zinc finger in the P box, you can now convert glucocorticoid receptor to that of estrogen receptor with respect to DNA binding specific is concerned.

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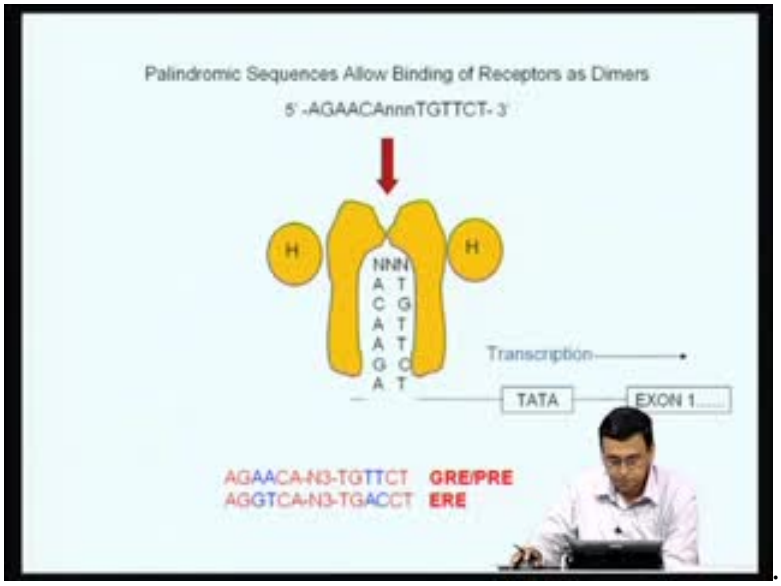

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So, if we simply mutate one amino acid glazing to glutamate instead of this receptor recognize glucocorticoid response element, it will go and recognize estrogen response element. So, you can see we have a number of steroid hormone receptor from the gross structure is the same. They all contain a zinc finger domain but minute amino acid differences between these receptors actually distinguish whether this was going to bind glucocorticoid response elements or whether go and bind to a estrogen response element. So, palindromic and all the steroid hormone response elements exists as palindromic

sequences and the receptor actually goes and binds as a dimer as shown here. One hormoner comes and binds one half side and another hormoner comes and binds another half side. That is how there is a bind dimer to the target DNA sequence and activates transcription and as I mentioned here the glucocorticoid response element and estrogen response element differ from each other from two nucleotides if is AGAACA half side is a glucocorticoid response element if the AGGTCA half side it becomes estrogen response element. If you just make one amino acid mutation within a DNA binding domain of glucocorticoid receptor instead of recognizing this sequence, now the glucocorticoid receptor recognize this sequence.

So, you can see how beautifully the nature has evolved target genes specific of this steroid hormone receptors. Now, one can ask question, you have glucocorticoid receptor, mineralocorticoid receptor and androgen receptor, they are all recognised the same sequences, then how is this specifically brought about because the mineralocorticoid physiological effects are different and glucocorticoid physiological effects are different. Androgen receptor physiological effects are different but they all are binding the same sequence.

So, how is that specific stage brought about? How specific physiological response are brought about by these different receptor, despite the fact that DNA binding by same sequence. So, the answer is although many receptors like GR, MR and AR, they all recognize with the same sequence, the specificity achieved by what kind of receptors actually present. For example, a tissue which is responsible to glucocorticoids may only have glucocorticoid tissue which is responsible. Glucocorticoids may have only glucocorticoid. It may not have a mineralocorticoid receptor, therefore it become responsible for glucocorticoid receptor.

So, by expressing specific receptors in this target types, they can achieve specific target gene specific receptor. So, very rarely you will find both the PR and GR may be expressed in the same type of cells. Even they are expressed in the same types of cells; the co-activators may require for the activation may be present in one receptor but not the other. We discuss this later in the lectures series.

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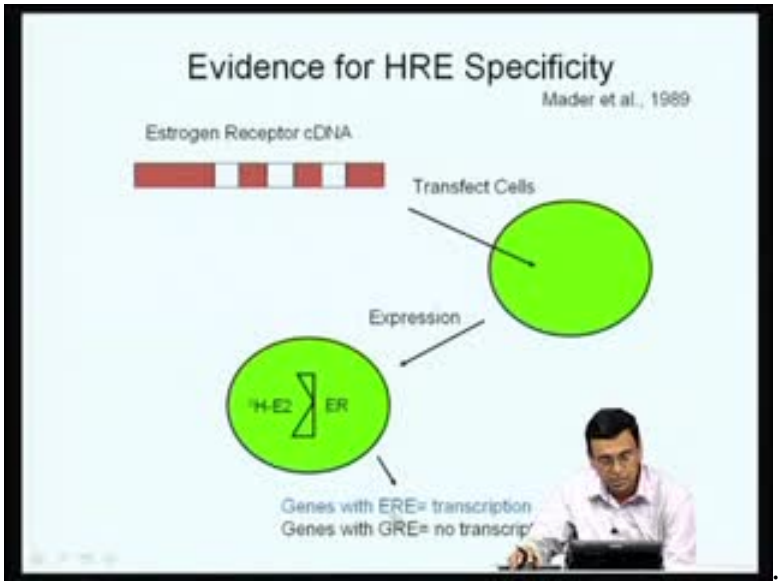

So, although the target genes specifically may be similar depending up on the cell type and tissue specific expression of the receptors as well as the different coordinated requirements like different gene may be activated and different receptor may be activated in different cells.

So, these kinds of experiments have been actually done again by very nice experiment where you can actually demonstrate this kind of specificities by actually, for example in the estrogen receptor, how do they demonstrate the receptor can actually activate from the estrogen response element but not from glucocorticoid response element in the same cell type. You can actually show you can take the estrogen receptor cDNA and transfer this in an expression that and transfer this expression into the cells of your interest when the glucocorticoid estrogen receptor is expressed. Now, if we had an estrogen hormone into this cell, it will now activate only those genes which can estrogens response element but it will not activate transcription of those genes containing a glucocorticoid response element. Indicating that these receptors binding into very specific hormone response elements, that is why they bring out transcription activation.

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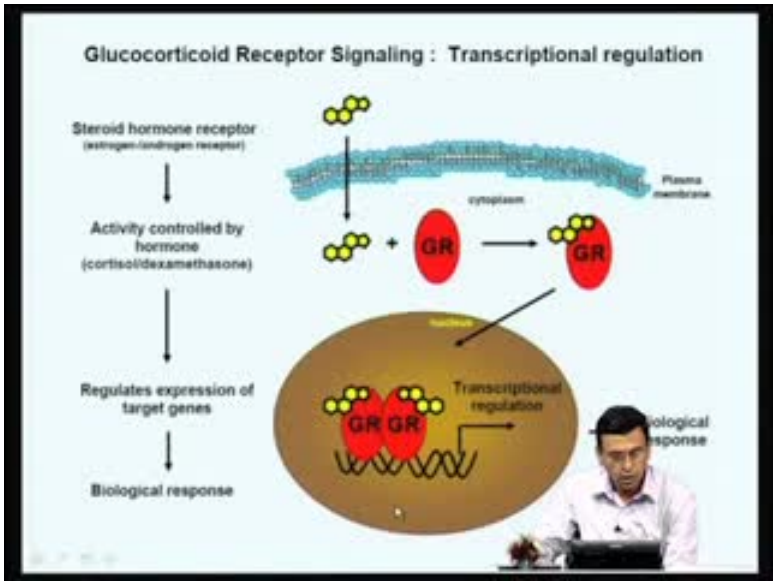

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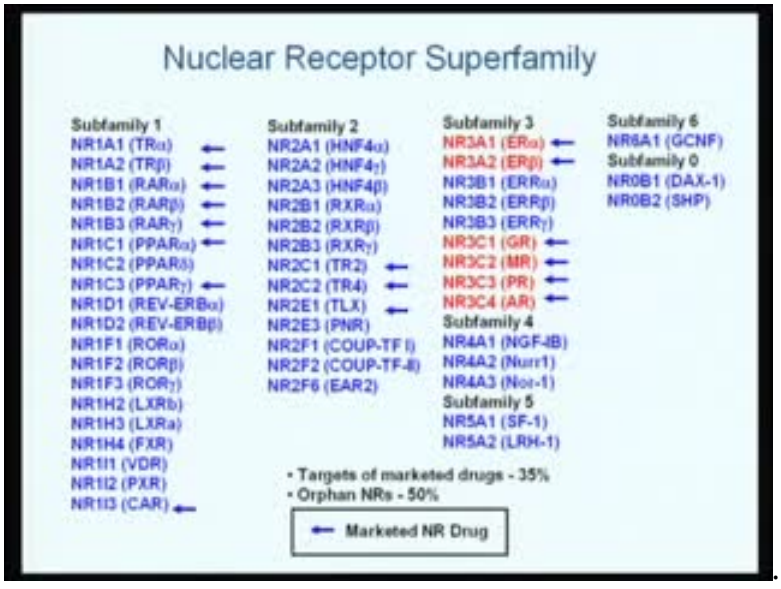

So, the identification of glucocorticoid receptors and disoffering the fact that this hormone go and binds to the receptor and then the receptor dimerize and they go and bind to specific hormone response element. The cloning of the glucocorticoid receptor cDNA as well as estrogen DNA are one of the hallmarks or land marks in the area of steroid hormone receptor signal transaction pathways. So, this marked a very important beginning in the understanding.

How this hormone functions? This actually led foundation for understanding the functions of many other receptors which we will discuss in the next few classes. Now, what we discussed today is just about one class of receptor, what are called as type 1 receptors and the estrogen receptors, glucocorticoid receptors, mineralocorticoid receptor, progesterone and androgen receptor. Now, there is no way huge family which is called as a nuclear receptor super family and the cloning of these cDNA's and demonstration that all this receptors actually have similar domain structure and then people started using the zinc finger cDNA binding domains of the receptors has proofs and using this proofs, they started screening cDNA libraries of various tissues.

They found that this kind of a domain structure is present number of other receptors and this led to what is known as a nuclear receptor super family and is a new nomenclature. This nuclear receptor family includes not only the steroid hormone receptors; it also includes thyroid hormone receptor, retinoid acid receptor, what is called as a PPAR and so on and so forth. In the next few classes, we will discuss some of these receptors and ask some question what is their importance and how do they activate transcription. So, the characterization of the cloning of the steroid hormone receptors is one of the very important land marks in the area of the nuclear receptor biology that actually paved way for the characterization of a number of other receptors which is mentioned here. So, nuclear receptor super family consists of not only the steroid hormone receptors marked in red but also number of other receptors.

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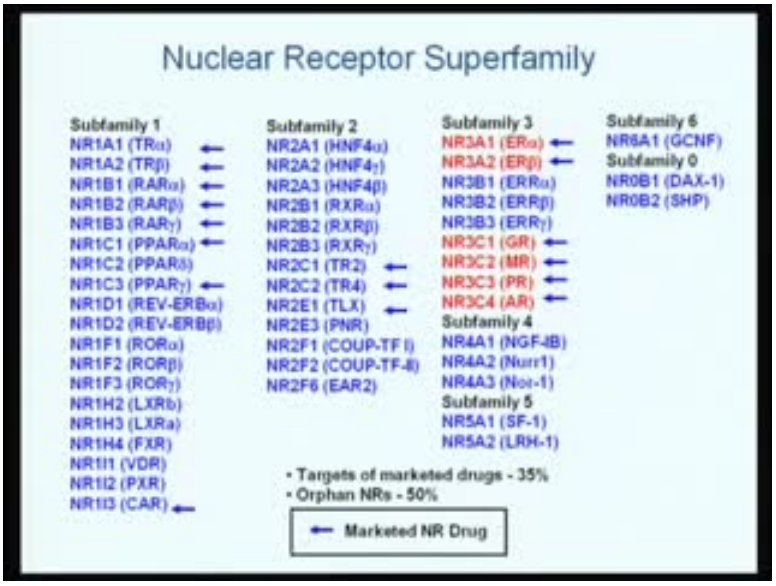

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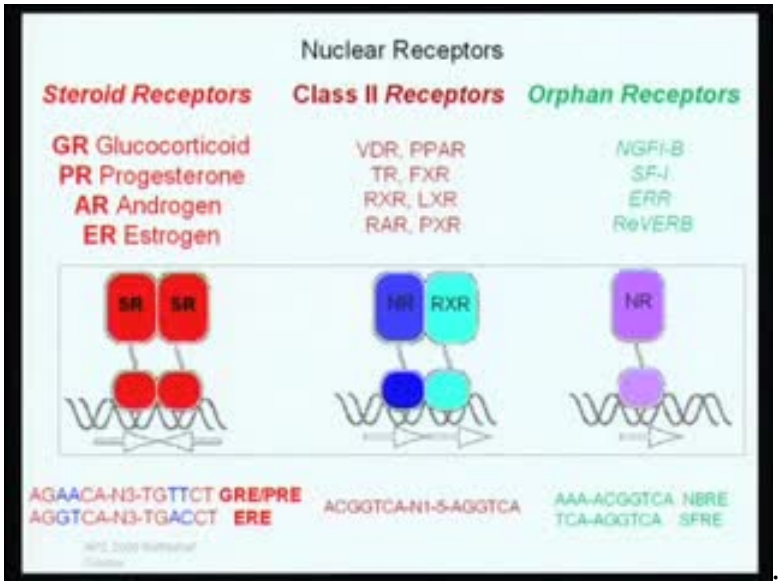

What is very interesting is that many of these receptors actually we know what kind of ligands bind but there are many other receptors; we do not know what kind of ligands they actually bind. For example, based on this the nuclear receptor can actually be classified into three different classes. One is the class 1 receptors which we have already discussed named steroid hormone receptors which actually bind to palindromic sequences or inverted repeat sequences and they are usually present in cytoplasm in the hormone and they are going to the nucleus in the hormone.

Tomorrow, in next class we are going to discuss the class 2 receptors which contain the receptor for a vitamin D thyroid hormones and retinoid acid and ask the question. How understanding glucocorticoid receptor function has paved way for the function of this receptors and what kind of sequences these receptors bind. They are also called what is called as orphan receptor that is these receptors share the same homology with that of the other receptors. They also contain zinc. They are also zinc finger transcription factors but we do not know what the ligands for these receptors are. Some of them actually functions of ligand independent transcription factors and they also bind to the different sequences.

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So, what I have tried to mention in this class is to demonstrate the discovery of steroid hormone receptors and how the discovery of steroid hormone receptor paved way for discovery of the other members of the nuclear super family. What I have shown in this slide, these three important people who actually made a wonderful contribution for this steroid hormone receptor biology. One is Pierre Chambon in France, Ronald Evans in Salk Institute, California and Elwood Jenson who actually made very pioneering work before cloning of the receptors actually began and recognizing their important contribution to this field they were actually awarded called the Albert Lasker basic medical research award. This often refers as the American noble prize and it is many people working in this era believe that these people actually may deserve or actually deserve noble prize and may be awarded noble prize in very near in the future because they made very important contribution for the discovery of the super family of nuclear hormone receptors and how this nuclear hormone receptors important for a number of physiological processes.

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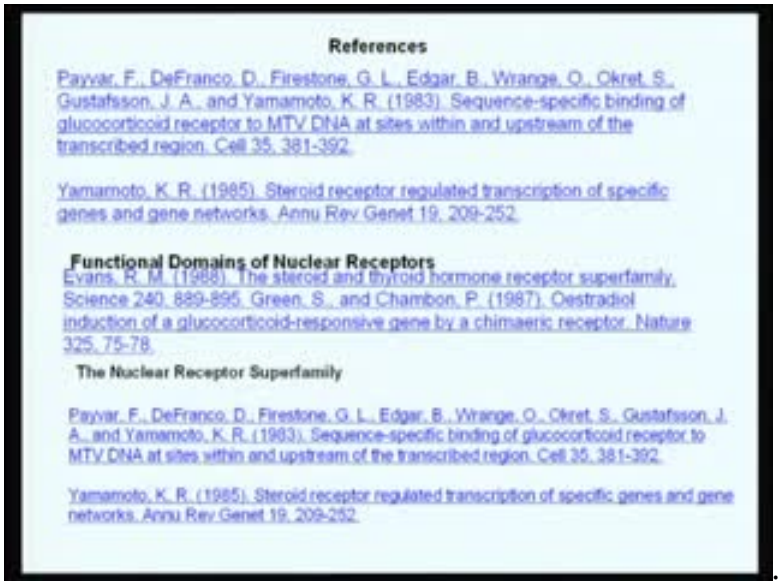

I had a privilege of working in Ron Evan lab and so I was working in this lab for three years and what we worked is about what is called cross coupling of nuclear receptors with others in the transcription factors and we will discuss that later in this course. So, what are the people who made a very important contribution had a privilege of working with him. I just listed some of the key references which are listed here which made a very important what are called land mark papers, how receptors were cloned and how they were identified and if you go through some of this land mark papers, you will understand and appreciate some of the efforts that has gone into this discovery of steroid hormone signaling. I think I will stop here.